UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

August 24, 2005

MEMORANDUM

Subject: Efficacy Review for DisCide Ultra Disinfecting Spray, EPA Reg. No. 10492-5; DP Barcode: D318432

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Applicant: Palmero Health Care
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Formulation from the Label:

Active Ingredient(s)	% by wt.
n-alkyl (60% C_{14}, 30% C_{16}, 5% C_{12}, 5% C_{18}) dimethyl benzyl ammonium chlorides	0.12 %
n-alkyl (68% C_{12}, 32% C_{14}) dimethyl ethyl benzyl ammonium chlorides	0.12 %
Isopropyl alcohol	63.25 %
Other Ingredients	36.51 %
Total	100.00 %

I. BACKGROUND

The product, DisCide Ultra Disinfecting Spray (EPA Reg. No. 10492-5), is an EPA-approved disinfectant with bactericidal, tuberculocidal, virucidal, herpecidal, and fungicidal effects, for use on hard, non-porous surfaces in institutional, household, commercial, and hospital or medical environments. The applicant requested an amendment to the registration of this product to add disinfectant claims for effectiveness against Hepatitis C Virus, Human Coronavirus, and one-minute contact time for *Mycobacterium bovis*. Study was conducted at Microbiotest, Inc., located at 105 Carpenter Drive, Sterling, VA 20164, and BioScience Laboratories, Inc., located at 30.

This data package contained a letter from the applicant's representative to EPA (dated May 19, 2005), EPA Form 8570-4 (Confidential Statement of Formula), EPA Form 8570-35 (Data Matrix), Eight studies (MRID No. 465695-01and MRID Nos. 465534-02 through 465534-08), Statements of No Data Confidentiality Claims for all eight studies, and the proposed label.

II. USE DIRECTIONS

The product is designed to be used for disinfecting hard, non-porous surfaces such as door handles, clean-up carts, light switches, sinks, tubs, tiles, toilets, shower doors, floors, dressing or linen carts, hampers, diaper pails, toilet seats, bed pans, plastic mattress covers, and lockers. Directions on the proposed label provided the following information regarding use of the product as a disinfectant: Clean surfaces prior to application. Thoroughly wet surface with DisCide Ultra Disinfecting Spray and allow to remain wet for 1 minute. When used as a fungicidal disinfectant, preclean the surfaces to be disinfected.

The proposed label directions also included special instructions for cleaning and decontaminating against HIV-1, HBV, and HCV on pre-cleaned surfaces or objects previously soiled with blood/body fluids. Finally, the label directions noted that: "This product is not to be used as a terminal sterilant/high level disinfectant on any surface or instrument . . .."

III. AGENCY STANDARDS FOR PROPOSED CLAIMS

Disinfectants for Use in Hospital or Medical Environments; Confirmatory Efficacy Data Requirements: Under certain circumstances, an applicant is permitted to rely on previously submitted efficacy data to support an application or amendment for registration of a product and to submit only minimal confirmatory efficacy data on his own product to demonstrate his ability to produce an effective formation. This includes a minor formulation change (e.g., a change in an inert ingredient) in a registered product. Confirmatory data must be developed on the applicant's own finished product. For hospital disinfectants, 10 carriers on each of 2 samples representing 2 different product lots must be tested against *Salmonella choleraesuis* (ATCC 10708), *Staphylococcus aureus* (ATCC 6538), and *Pseudomonas aeruginosa* (ATCC 15442) using either the AOAC Use-Dilution Method or the AOAC Germicidal Spray Products as Disinfectants Method. **Performance requirements:** Killing on all carriers is required. These Agency standards are presented in DIS/TSS-5.

Disinfectants for Use as Tuberculocides (Using the AOAC Tuberculocidal Activity Test Method or the AOAC Germicidal Spray Products Test Method)

Disinfectants may bear additional label claims of effectiveness as tuberculocides when supported by appropriate tuberculocidal effectiveness data. Certain chemical classes (i.e.,

glutaraldehyde and quaternary ammonium compounds) are required to undergo validation testing in addition to basic testing. Products that are formulated with other chemical groups do not require validation testing. Products may be tested using one of four recommended methods: the AOAC Tuberculocidal Test Method, Tuberculocidal Activity of Disinfectants Test Method with significant modification of the standard test conditions of contact time and/or temperature, Quantitative Tuberculocidal Activity Test Method, and AOAC Germicidal Spray Products Test Method.

When using the existing or modified AOAC Tuberculocidal Activity Test Methods, or the AOAC Germicidal Spray Products Test Method, ten (10) carriers for each of two samples, representing two different batches of product, must be tested against *Mycobacterium bovis* BCG (a member of the *Mycobacterium tuberculosis* species complex). When using the existing or modified AOAC Tuberculocidal Activity Test Method, or the AOAC Germicidal Spray Products Test Method, killing on all carriers/slides as demonstrated in Modified Proskauer-Beck Broth, and no growth in any of the inoculated tubes of two additional media (i.e., Middlebrook 7H9 Broth Difco B, Kirchners Medium, and/or TB Broth Base) is required. Agency standards are presented in EPA DIS/TSS-6, Subdivision G Guidelines, and "EPA Data Call-in Notice for Tuberculocidal Claims," dated June 13, 1986.

Disinfectants for Use as Fungicides (Against Pathogenic Fungi): Effectiveness of liquid disinfectants against specific pathogenic fungi must be supported by efficacy data derived from each of 2 samples representing 2 different batches using the AOAC Fungicidal Test. **Performance standard**. The highest dilution that kills all fungal spores is the minimum effective concentration.

Alternatively, the AOAC Use Dilution Method, modified to conform with appropriate elements in the AOAC Fungicidal Test, may be employed. If the product is intended for use as a spray, the AOAC Germicidal Spray Products Test must be employed. The inoculum in the above tests must be modified to provide a concentration of at least 10^6 conidia per carrier. Ten carriers on each of 2 samples representing 2 different batches must be employed in the test. **Performance requirements:** Killing of the test microorganism on all carriers is required. The above Agency standards are presented in DIS/TSS-06.

Note: As an interim policy, the Agency is accepting studies with dried carrier counts that are at least 10^4 for *Trichophyton mentagrophytes* and *Aspergillus niger*. The Agency recognizes laboratories are experiencing problems in maintaining dried carrier counts at the 10^6 level. This interim policy will be in effect until the Agency determines that the laboratories are able to achieve consistent carrier counts at the 10^6 level.

Virucidal requirements: The effectiveness of virucides against specific viruses must be supported by efficacy data that simulates, to the extent possible in the laboratory, the conditions under which the product is intended to be used. Carrier methods that are modifications of the AOAC Use-Dilution Method (for liquid disinfectants) must be used in developing data for virucides intended for use upon dry inanimate, environmental surfaces (e.g., floors, tables, cleaned dried medical instruments). To simulate in-use conditions, the specific virus to be treated must be inoculated onto hard surfaces, allowed to dry, and then treated with the product according to the directions for use on the product label. One surface for each of 2 different batches of disinfectant must be tested against a recoverable virus titer of at least 10^4 from the test surface for a specified exposure period at room temperature. Then, the virus must be assayed by an appropriate virological technique, using a minimum of four determinations per each dilution assayed. Separate studies are required for each virus. The calculated viral titers must be reported with the test results. **Performance standard:** For the data to be considered

acceptable, results must demonstrate complete inactivation of the virus at all dilutions. When cytotoxicity is evident, at least a 3-log reduction in titer must be demonstrated beyond the cytotoxic level. These Agency standards are presented in DIS/TSS-7.

Virucides - Use of a Surrogate Virus: For certain viruses, there are no *in vitro* systems or *in vivo* animal models (except for humans and chimpanzees). The Agency permits the testing of surrogate viruses in these cases, for example, Bovine viral diarrhea virus as a surrogate for human Hepatitis C virus, Duck Hepatitis B virus as a surrogate for Human Hepatitis B virus, and Feline calicivirus as a surrogate for Norwalk virus. When a surrogate virus is used, confirmatory data must be developed by an independent laboratory on at least 1 product lot.

Supplemental Recommendations: An antimicrobial agent identified as a "one-step" cleaner-disinfectant, cleaner-sanitizer, or one intended to be effective in the presence of organic soil must be tested for efficacy with an appropriate organic soil load, such as 5% blood serum. The organic soil level suggested is considered appropriate for simulating lightly or moderately soiled surface conditions. When the surface to be treated has heavy soil deposits, a cleaning step must be recommended prior to application of the antimicrobial agent. The effectiveness of antimicrobial agents must be demonstrated in the presence of a specific organic soil at an appropriate concentration level when specifically claimed and/or indicated by the pattern of use. The hard water tolerance level may differ with the level of antimicrobial activity (e.g., sanitizer vs. disinfectant) claimed. To establish disinfectant efficacy in hard water, all microorganisms (i.e., bacteria, fungi, viruses) claimed to be controlled must be tested by the appropriate Recommended Method at the same hard water tolerance level. All products tested by the recommended methods may be tested at the exposure periods prescribed in those methods. When an antimicrobial agent is intended to be effective in treating a non-porous surface, the Recommended Methods simulate this condition by using non-porous surface carrier (stainless steel cylinder or glass slide) specified in the method. The exposure period or manner of use necessary to provide efficacy must be featured prominently on the product label. These Agency standards are presented in DIS/TSS-2

IV. BRIEF DESCRIPTION OF THE DATA

1. MRID 465672-01 "Hard Surface Disinfection Evaluation For One Spray Product Versus Four Microorganism Species" for DisCide Ultra Disinfecting Spray, by Jennifer Jill Lawrence; Project number: 040713-204. Study conducted at Bioscience Laboratories, Inc. Study completed on January 31, 2005.

This study was conducted against *Pseudomonas aeruginosa* (ATCC # 15442), *Salmonella choleraesuis* (ATCC # 10708), *Staphylococcus aureus* (ATCC # 6538), and *Trichophyton mentagrophytes* (ATCC # 9533). Two lots (Lot Nos. 14-05184A and 14-07204B) of the product, DisCide Ultra Disinfecting Spray, were tested using AOAC Official Method 961.02, Germicidal Spray Products as Disinfectants (17th Edition, 2000) (Bioscience Laboratories, Inc.'s protocol # 040713-204, copy provided). The product was received ready-to-use. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Sterile glass slides carriers were contaminated with 0.01 ml of 48hrs (bacteria) or 16 days (*T. mentagrophytes*) old broth culture of the test organism. The carriers were dried for 40 minutes at 35±2°C. Ten carriers were tested per organism and per lot. Each carrier was sprayed with the product from a distance of 12 inches until thoroughly wet. The carriers were allowed to remain wet for 1 minute at 20°C. Excess liquid was allowed to drain and the carriers were

transferred to tubes of Letheen Broth (Bacteria) or Glucose Broth with Letheen and Tween 80 (*T. mentagrophytes*) for neutralization and subculture. Tubes were incubated for 48 hours at 35±2°C (bacteria) or 7 days at 25±2°C (fungi) and examined for the presence or absence of visible growth. Controls included those for carrier counts, viability, neutralizer effectiveness, sterility, and confirmation of the challenge microorganism. The reported average colony forming units (CFU) per dried carrier, for each test microorganism, are as follows: ***Pseudomonas aeruginosa*** **2.1 x 10^6**, ***Staphylococcus aureus*** **2.8 x 10^6**, ***Salmonella choleraesuis*** **3.1 x 10^4**, and ***Trichophyton mentagrophytes*** **3.0 x 10^5**.

Note: Protocol **deviation** reported in the study was reviewed and found to be acceptable.

2. MRID 465534-02 "Germicidal Spray Test" for DisCide Ultra Disinfecting Spray, by Angela L. Hollingsworth; Project number: 552-108. Study conducted at Microbiotest, Inc. Study completed on March 23, 2005.

This study was conducted against *Escherichia coli* (ATCC 11229). Two lots (Lot Nos. 14-07204A and 14-09014A) of the product, DisCide Ultra Disinfecting Spray, were tested using Microbiotest protocol 552.3.01.27.05 (copy provided). The product was received ready-to-use. Heat-inactivated horse serum was added to the culture to achieve a 5% organic soil load. Ten (10) glass slide carriers per lot were inoculated with 0.01-0.03 ml of a 48-54 hour old suspension of the test organism. The carriers were dried for 20-40 minutes at 37±2°C. Each carrier was sprayed with the product from a distance of 12 inches until thoroughly wet. The carriers were allowed to remain wet for 1 minute at 20°C. Excess liquid was allowed to drain and the carriers were transferred to tubes of Letheen Broth containing 7% Polysorbate 80 and 1% Lecithin (RB+) to neutralize. All subcultures were incubated for 48±2 hours at 37±2°C, and then examined for the presence or absence of visible growth. Controls included viability, dried carrier counts, bacteriostasis, neutralizer effectiveness, confirmation of challenge organism, and sterility. The reported average colony forming units per carrier, for the test microorganism, is: ***Escherichia coli*** **7.5 x 10^5**.

3. MRID 465534-03 "Germicidal Spray Test" for DisCide Ultra Disinfecting Spray, by Angela L. Hollingsworth; Project number: 552-106. Study conducted at Microbiotest, Inc. Study completed on March 23, 2005.

This study was conducted against *Enterococcus faecium,* Vancomycin resistant (VRE) (ATCC 51559). Two lots (Lot Nos. 14-07204A and 14-09014A) of the product, DisCide Ultra Disinfecting Spray, were tested using Microbiotest protocol 552.1.01.27.05 (copy provided). The product was received ready-to-use. Heat-inactivated horse serum was added to the culture to achieve a 5% organic soil load. Ten (10) glass slide carriers per lot were inoculated with 0.01-0.03 ml of a 48-54 hour old suspension of the test organism. The carriers were dried for 20-40 minutes at 37±2°C. Each carrier was sprayed with the product from a distance of 12 inches until thoroughly wet. The carriers were allowed to remain wet for 1 minute at 20°C. Excess liquid was allowed to drain and the carriers were transferred to tubes of Letheen Broth containing 7% Polysorbate 80 and 1% Lecithin (RB+) to neutralize. All subcultures were incubated for 48±2 hours at 37±2°C, and then examined for the presence or absence of visible growth. Controls included viability, dried carrier counts, bacteriostasis, neutralizer effectiveness, confirmation of challenge organism, and sterility. The reported average colony forming units per carrier, for the test microorganism, is: ***Enterococcus faecium*** **(VRE) 5.5 x 10^5**.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

4. MRID 465534-04 "Germicidal Spray Test" for DisCide Ultra Disinfecting Spray, by Angela L. Hollingsworth; Project number: 552-107. Study conducted at Microbiotest, Inc. Study completed on March 23, 2005.

This study was conducted against *Staphylococcus aureus* (MRSA) (ATCC 33592). Two lots (Lot Nos. 14-07204A and 14-09014A) of the product, DisCide Ultra Disinfecting Spray, were tested using Microbiotest protocol 552.2.01.27.05 (copy provided). The product was received ready-to-use. Heat-inactivated horse serum was added to the culture to achieve a 5% organic soil load. Ten (10) glass slide carriers per lot were inoculated with 0.01-0.03 ml of a 48-54 hour old suspension of the test organism. The carriers were dried for 20-40 minutes at 37±2°C. Each carrier was sprayed with the product from a distance of 12 inches until thoroughly wet. The carriers were allowed to remain wet for 1 minute at 20°C. Excess liquid was allowed to drain and the carriers were transferred to tubes of Letheen Broth containing 7% Polysorbate 80 and 1% Lecithin (RB+) to neutralize. All subcultures were incubated for 48±2 hours at 37±2°C, and then examined for the presence or absence of visible growth. Controls included viability, dried carrier counts, bacteriostasis, neutralizer effectiveness, confirmation of challenge organism, and ***Staphylococcus aureus*** **(MRSA) 3.9 x 10^5**.

5. MRID 465534-05 "Tuberculocidal Activity of a Germicidal Spray" for DisCide Ultra Disinfecting Spray, by Angela L. Hollingsworth; Project number: 552-102. Study conducted at Microbiotest, Inc. Study completed on January 5, 2005.

This study was conducted against *Mycobacterium bovis* - BCG (Organon Teknika). Two lots (Lot Nos. 14-07204A and 14-09014A) of the product, DisCide Ultra Disinfecting Spray, which contains quaternary ammonium chlorides and isopropyl alcohol, were tested using Microbiotest protocol PAL.2.08.26.04 (copy provided). The product was received ready-to-use. Heat-inactivated horse serum was added to the culture to achieve a 5% organic soil load. Sterile glass microscope slides carriers were contaminated with 0.01-0.03 ml of 14-25 days old broth culture of the test organism. The carriers were dried for 20-40 minutes at 37±2°C. For each product lot, 10 contaminated carriers were individually sprayed with the product from a distance of 12 inches until thoroughly wet. The carriers were allowed to remain wet for 1 minute at 20°C. Excess liquid was allowed to drain and the carriers were transferred to tubes of Modified Proskauer Beck Medium containing 7% Polysorbate 80 and 1% Lecithin (MPBM+) to neutralize. Each carrier was shaken and transferred to a tube containing 20 ml of MPBM. From each tube of neutralizer, 2 ml aliquots were subcultured to individual tubes containing 20 ml of Middlebrook 7H9 Broth and 2 ml aliquots were subcultured to individual tubes containing 20 ml of Kirchner Medium. All tubes used for primary and secondary transfers were incubated for 60 days at 37±2°C and examined for the presence or absence of visible growth. All plates were incubated for 14-25 days at 37±2°C. When no growth was observed, culture tubes were incubated an additional 30 days. Controls included those for carrier counts, viability, neutralizer effectiveness, sterility, and confirmation of the challenge microorganism. The reported average Colony Forming Units (CFU) per carrier, for the test microorganism, is: ***Mycobacterium bovis*** **BCG 3.6 x 10^4**.

Note: Protocol amendments reported in the study were reviewed and found to be acceptable.

6. MRID 465534-06 "Virucidal Effectiveness Test Using Bovine viral diarrhea virus (BVDV) (Surrogate for human Hepatitis C virus)" for DisCide Ultra Disinfecting

Spray, by M. Khalid Ijaz. Study conducted at MicroBioTest, Inc. Study completion date – December 09, 2004. Laboratory Project Identification Number 552-104.

This study, under the direction of Study Director M. Khalid Ijaz, was conducted against the Bovine viral diarrhea virus (strain not specified; obtained from American BioResearch Laboratories) using MDBK cells (ATCC CCL-22) as the host system. The study protocol followed MicroBioTest Protocol "Virucidal Effectiveness Test Using Bovine viral diarrhea virus (Surrogate for human Hepatitis C virus)," dated August 26, 2004 (copy provided). Two lots (Lot Nos. 14-07204A and 14-09014A) of the product, DisCide Ultra Disinfecting Spray, were tested. The product was received ready-to-use. The inoculum contains at least 5% organic soil load. Two glass carriers were tested for each product lot against the target virus. Films of virus were made by spreading 0.2 ml of stock virus on the bottoms of separate sterilized glass Petri dishes. The virus films were dried for 30-60 minutes at room temperature. For each lot of product, carriers were individually sprayed until thoroughly wet, for four seconds at a distance of 12 inches from the test surface. After one-minute contact period at 22°C, the test agent was neutralized with 2.0 ml of horse serum and the mixture was scraped from the surface of the dish with a cell scraper. Each sample (0.5 ml) was loaded onto pre-spun Sephacryl columns and spun to obtain the eluate. Ten-fold serial dilutions were prepared, using Minimum Essential Media Eagle's containing 5% horse serum. MDBK cells were inoculated in quadruplicate with an unspecified amount of each dilution and incubated at 37±2°C in 5±1% CO_2 for 3-5 days. The plates were assayed by direct immunofluorescence assay. Host cells were fixed with alcohol, stained and read for infectivity, and enumerated as Most Probable Number (MPN). Controls included those for cell viability, virus stock titer, plate recovery, column titer, neutralizer effectiveness, cytotoxicity, cytotoxicity-related viral interference, and data consistency. Lonza Inc.'s Bardac 2280 (Lot No. F42223357) was used as the data consistency control at two concentrations, 50±5 ppm and 350±15 ppm. The titer of the dried virus control was **6.4826 $\log_{10}$**. Taking the cytotoxicity and neutralization control results into consideration, the reduction in viral titer was ≥**4.1026 $\log_{10}$** for both batches.

7. MRID 465534-07 "Confirmatory Virucidal Effectiveness Test Using Bovine viral diarrhea virus (BVDV) (Surrogate for human Hepatitis C virus)" for DisCide Ultra Disinfecting Spray, by Zheng Chen. Study conducted at MicroBioTest, Inc. Study completion date – December 13, 2004. Laboratory Project Identification Number 552-105.

This study, under the direction of Study Director Zheng Chen, was conducted against the Bovine viral diarrhea virus (strain not specified; obtained from American BioResearch Laboratories) using MDBK cells (ATCC CCL-22) as the host system. The study protocol followed MicroBioTest Protocol "Virucidal Effectiveness Test Using Bovine viral diarrhea virus (Surrogate for human Hepatitis C virus)," dated August 26, 2004 (copy provided). One lot (Lot Nos. 14-09014A) of the product, DisCide Ultra Disinfecting Spray, was tested. The product was received ready-to-use. The inoculum contains at least 5% organic soil load. Two glass carriers of target virus were tested. Films of virus were made by spreading 0.2 ml of stock virus on the bottoms of separate sterilized glass Petri dishes. The virus films were dried for 30-60 minutes at room temperature. For each lot of product, carriers were individually sprayed until thoroughly wet, for four seconds at a distance of 12 inches from the test surface. After one-minute contact period at 22°C, the test agent was neutralized with 2.0 ml of horse serum and the mixture was scraped from the surface of the dish with a cell scraper. Each sample (0.5 ml) was loaded onto pre-spun Sephacryl columns and spun to obtain the eluate. Ten-fold serial dilutions were prepared, using Minimum Essential Media Eagle's containing 5% horse serum. MDBK cells were inoculated in quadruplicate with an unspecified amount of each dilution and incubated at

37±2°C in 5±1% CO_2 for 3-5 days. The plates were assayed by direct immunofluorescence assay. Host cells were fixed with alcohol, stained and read for infectivity, and enumerated as Most Probable Number (MPN). Controls included those for cell viability, virus stock titer, plate recovery, column titer, neutralizer effectiveness, cytotoxicity, cytotoxicity-related viral interference, and data consistency. Lonza Inc.'s Bardac 2280 (Lot No. F42223357) was used as the data consistency control at two concentrations, 50±5 ppm and 350±15 ppm. The titer of the dried virus control was **6.37986 $\log_{10}$**. Taking the cytotoxicity and neutralization control results into consideration, the reduction in viral titer was ≥**4.0 $\log_{10}$** for the batch.

8. MRID 465534-08 "Virucidal Efficacy Test, Human Coronavirus" for DisCide Ultra Disinfecting Spray, by M. Khalid Ijaz. Study conducted at MicroBioTest, Inc. Study completion date – December 09, 2004. Laboratory Project Identification Number 552-103.

This study was conducted against Human Coronavirus (ATCC VR-740), using MRC-5 cells (human embryonic lung cells; Diagnostic Hybrids, Inc., Athens, OH) as the host system. Two lots (Lot Nos. 14-07204A and 14-09014A) of the product, DisCide Ultra Disinfecting Spray, were tested. The study protocol followed MicroBioTest Protocol "Virucidal Efficacy Test, Human Coronavirus," dated August 26, 2004 (copy provided). The product was received ready-to-use. The stock virus culture contained a 5% fetal bovine serum. Films of virus were prepared by spreading 0.2 ml of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were dried at room temperature for 30-60 minutes. For each lot of product, separate dried virus films were sprayed for 4 seconds at a distance of 12 inches from the carrier surface. The virus films remained exposed to the use solution for 1 minute at 22°C. After exposure, 2.0 ml of fetal bovine serum was added to neutralize. The plates were scraped with a cell scraper to re-suspend the contents. The neutralized mixture was passed through a Sephacryl column, and diluted serially in Minimum Essential Medium supplemented with 10% fetal bovine serum. MRC-5 cells in multi-well culture dishes were inoculated in quadruplicate with 0.2 ml of the dilutions. The cultures were incubated at 33±1°C in a humidified atmosphere of 5±1% CO_2 and scored periodically for 10-14 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for toxicity, toxicity-related viral interference, plate recovery, column titer, virus stock titer, host viability, and neutralizer effectiveness. The 50% cell culture infections dose per ml ($CCID_{50}$/ml) was determined using the method of Reed and Muench. The titer of the plate recovery control was **5.50 $\log_{10}$**. Taking the cytotoxicity and neutralization control results into consideration, the reduction in viral titer was ≥**3.0 $\log_{10}$** for both batches.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

V. RESULTS

MRID Number	Organism	No. Exhibiting Growth/Total No. Tested		Carrier Count (CFU/carrier)
		Lot No. 14-05184A	Lot No. 14-07204B	
465534-01	*Pseudomonas aeruginosa*	0/10	0/10	2.1×10^6
	Salmonella choleraesuis	0/10	0/10	3.1×10^4
	Staphylococcus aureus	0/10	0/10	2.8×10^6
	Trichophyton mentagrophytes	0/10	1/10	3.0×10^5

MRID Number	Organism	Subculture Medium	No. Exhibiting Growth/Total No. Tested		Carrier Count (CFU/carrier)
			Lot No. 14-07204A	Lot No. 14-09014A	
465534-02	*Escherichia coli*	RB+	0/10	0/10	7.5×10^5
465534-03	*Enterococcus faecium* (VRE)	RB+	0/10	0/10	5.5×10^5
465534-04	*Staphylococcus aureus* (MRSA)	RB+	0/10	0/10	3.9×10^5
465534-05	*Mycobacterium bovis* BCG	Neutralizer	0/10 60 days 0/10 90 days	0/10 60 days 0/10 90 days	3.6×10^4
		Modified Proskauer Beck Medium	0/10 60 days 0/10 90 days	0/10 60 days 0/10 90 days	
		Middlebrook 7H9 Broth	0/10 60 days 0/10 90 days	0/10 60 days 0/10 90 days	
		Kirchner's Medium	0/10 60 days 0/10 90 days	0/10 60 days 0/10 90 days	

MRID Number	Organism	Mean of Duplicates expressed in Log_{10}MPN/ml				
		Lot No. 14-07204A	Lot No. 14-09014A	Plate Recovery Control	Bardac	
					50 ppm	350 ppm
465534-06	Bovine viral diarrhea virus	≤2.38	≤2.38	6.4826	3.7825	0.00
465534-07	Bovine viral diarrhea virus	---	≤2.3798	6.3798	3.7825	0.00

MRID Number	Organism	Results			Dried Virus Control ($CCID_{50}$/ ml)
			Lot No. 14-07204A	Lot No. 14-09014A	
465534-08	Human Coronavirus	10^{-2} dilution	Cytotoxicity	Cytotoxicity	$10^{5.50}$
		10^{-3} to 10^{-8} dilutions	Complete inactivation	Complete inactivation	
		$TCID_{50}$/ ml	$\leq 10^{2.5}$	$\leq 10^{2.5}$	
		Log reduction	≥ 3.0 $\log_{10}$	≥ 3.0 $\log_{10}$	

VI. CONCLUSIONS

1. The submitted efficacy data **support** the use of the product, Discide Ultra Disinfecting Spray, as a One-Step disinfectant with bactericidal activity against the following microorganisms on hard, non-porous surfaces in the presence of a 5% organic soil load for a contact time of 1 minute at 20.0°C.

Pseudomonas aeruginosa	MRID No. 465534-01
Salmonella choleraesuis	MRID No. 465534-01
Staphylococcus aureus	MRID No. 465534-01
Escherichia coli	MRID No. 465534-02
Enterococcus faecium (VRE)	MRID No. 465534-03
Staphylococcus aureus (MRSA)	MRID No. 465534-04

2. The submitted efficacy data (MRID No. 465534-05) **support** the use of the product, Discide Ultra Disinfecting Spray, as a One-Step disinfectant with tuberculocidal activity against *Mycobacterium bovis* BCG on hard, non-porous surfaces in the presence of a 5% organic soil load for a contact time of 1 minute at 20.0°C.

3. The submitted efficacy data (MRID No. 465534-01) **did not support** the use of the product, Discide Ultra Disinfecting Spray, as a One-Step disinfectant with fungicidal activity against *Trichophyton mentagrophytes* on hard, non-porous surfaces in the presence of a 5% organic soil load for a contact time of 1 minute at 20.0°C.

4. The submitted efficacy data **support** the use of the product, Discide Ultra Disinfecting Spray, as a One-Step disinfectant with virucidal activity against the following microorganisms on hard, non-porous surfaces in the presence of a 5% organic soil load for a contact time of 1 minute at 20.0°C.

Human Hepatitis C virus	MRID Nos. 465534-06 and 465534- 07
Human Coronavirus	MRID No. 465534-08

VII. RECOMMENDATIONS

1. The proposed label claims that the product, Discide Ultra Disinfecting Spray, is a One-Step disinfectant with bactericidal activity against the following microorganisms on hard, non-porous surfaces in the presence of a 5% organic soil load for a contact time of 1 minute at 20.0°C, **are supported** by the applicant's data: *Pseudomonas aeruginosa, Salmonella choleraesuis, Staphylococcus aureus, Escherichia coli, Enterococcus faecium* (VRE), and *Staphylococcus aureus* (MRSA).

2. The efficacy testing supports the proposed label claims that the use of the product, *Ultra-Cide Ready to Use*, is a One-Step disinfectant with virucidal activity against the following microorganisms on hard, non-porous surfaces in the presence of a 5% organic soil load for a contact time of 1 minute at 20.0°C, **are supported** by the applicant's data: Hepititis B Virus (HBV), Hepatitis C Virus (HCV), Human Coronavirus, HIV-1 (AIDS virus), Adenovirus type 2, and Herpes Simplex Virus type 2 (genital herpes virus).

3. The proposed label claims that the product, Discide Ultra Disinfecting Spray, is a One-Step disinfectant with tuberculocidal activity against *Mycobacterium bovis* BCG on hard, non-porous surfaces in the presence of a 5% organic soil load for a contact time of 1 minute at 20.0°C, **are supported** by the applicant's data.

4. The proposed label claims that the product, Discide Ultra Disinfecting Spray, is a disinfectant with fungicidal activity against *Trichophyton mentagrophytes* on hard, non-porous surfaces for a contact time of 1 minute at 20.0°C, **are supported** by the applicant's data.

5. The applicant must delete **leather**, on page 2 of the proposed label, from the list of non-porous surfaces.